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Developmentally regulated cleavage of tRNAs in the bacterium *Streptomyces coelicolor*

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ABSTRACT

The ability to sense and respond to environmental and physiological signals is critical for the survival of the soil-dwelling Gram-positive bacterium *Streptomyces coelicolor*. Nutrient deprivation triggers the onset of a complex morphological differentiation process that involves the raising of aerial hyphae and formation of spore chains, and coincides with the production of a diverse array of clinically relevant antibiotics and other secondary metabolites. These processes are tightly regulated; however, the genes and signals involved have not been fully elucidated. Here, we report a novel tRNA cleavage event that follows the same temporal regulation as morphological and physiological differentiation, and is growth medium dependent. All tRNAs appear to be susceptible to cleavage; however, there appears to be a bias towards increased cleavage of those tRNAs that specify highly utilized codons. In contrast to what has been observed in eukaryotes, accumulation of tRNA halves in *S. coelicolor* is not significantly affected by amino acid starvation, and is also not affected by induction of the stringent response or inhibition of ribosome function. Mutants defective in aerial development and antibiotic production exhibit altered tRNA cleavage profiles relative to wild-type strains.

INTRODUCTION

In the filamentous soil bacterium *Streptomyces coelicolor*, starvation conditions stimulate visibly detectable metabolic and morphological adaptations. Under nutrient replete conditions, *S. coelicolor* forms a vegetative mycelium, reminiscent of the filamentous fungi; however, starvation induces the development of reproductive aerial hyphae, collectively referred to as the aerial mycelium, which differentiate to form chains of exospores.

This morphological transition is associated with a shift from primary to secondary metabolism in the vegetative hyphae, and involves the production of pigmented antibiotics. The onset of both aerial hyphae formation and secondary metabolism is controlled in part by a class of genes termed the '*bld*' (for 'bald') genes, while the sporulation process is controlled by the '*whi*' (for 'white') genes. The majority of *bld* and *whi* genes encode regulatory proteins; however, neither their downstream targets nor the signal(s) that stimulate their expression/activity are well understood (1,2).

There are several disparate lines of evidence that connect the ability to sense nutritional conditions with the onset of differentiation. The inability of many *bld* mutants to raise an aerial mycelium is conditionally dependent upon the carbon-source available in their growth medium (3); these same *bld* mutants are also defective in their ability to regulate their carbon source utilization (4). More recently, aerial development has been shown to be inhibited by both extracellular N-acetylglucosamine (5) and high concentrations of external organic acids (6), while both aerial hyphae formation and antibiotic production are affected by nitrate limitation or amino acid starvation (7,8).

In many bacteria, the response to amino acid starvation is associated with the synthesis of the small molecule ppGpp by the ribosome-associated *relA* gene product. ppGpp accumulation is accompanied by global changes in gene expression and cellular physiology that are believed to enhance survival under suboptimal growth conditions; this phenomenon has been termed the 'stringent response' (9). In the streptomycetes, the stringent response is associated with a stimulation of antibiotic production and morphological differentiation, particularly under nitrogen-limiting conditions (8,10). Typically, the stringent response is initiated by an increase in the ratio of uncharged tRNAs to aminoacylated tRNAs. The resulting interaction of uncharged tRNAs with the A site of the 50S ribosome causes stalling of protein synthesis and a subsequent activation of RelA activity. Ultimately, this triggers both the downregulation of stable

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Table 1. *S. coelicolor* strains

<i>S. coelicolor</i> strain	Genotype	Reference
M145	SCP1– SCP2–	20
J1501	<i>hisA1 uraA1 strA1 pgl–</i> SCP1 [–] SCP2 [–]	18
C109	J1501 <i>bldH</i>	17
J660	<i>bldC18 mthB2 cysD18</i>	15
J669	<i>agaA1</i> SCP1 ^{NF} SCP2 [*] <i>bldB43 mthB2 cysD18</i>	15
J1700	<i>agaA1</i> SCP1 ^{NF} SCP2 [*] J1501 <i>bldA39</i>	14
J2400	M145 <i>whiG::hyg</i>	19
J2402	M145 <i>whiB::hyg</i>	19

RNA (rRNA, tRNA) synthesis and the upregulation of amino acid biosynthetic gene expression (11), although in *S. coelicolor*, recent work has revealed that many amino acid biosynthetic clusters are actually repressed in response to ppGpp (10).

Starvation conditions are also associated with the degradation of stable RNAs. This primarily affects rRNAs, and it is presumed that rRNA degradation slows protein synthesis, and at the same time releases nutrient stores (12). In contrast, tRNAs have been shown to be stable under starvation conditions (13). A number of factors contribute to the overall stability of tRNA molecules: extensive secondary and tertiary structure of the mature tRNAs mean they are less accessible to nucleases than other RNAs in the cell; the 3' termini of tRNAs are protected from exonucleases by aminoacylation; and charged tRNAs are often associated with ribosomes, elongation factors and acyl-tRNA synthetases, thus rendering them inaccessible to most ribonucleases. Despite their apparent stability under starvation conditions, those tRNAs most susceptible to degradation would be uncharged tRNAs that are not associated with the translation machinery. This tRNA sub-population would be most prevalent during conditions of nutrient deprivation, particularly amino acid starvation, and thus it may be possible to correlate tRNA degradation with the nutritional status of a cell. Here, we provide evidence for the accumulation of tRNA cleavage products, particularly tRNA halves, in a medium-dependent manner during the development of *S. coelicolor*.

MATERIALS AND METHODS

Streptomyces strains and culture conditions

Wild type *S. coelicolor* strain M145 and developmental mutants *bldA* (14), *bldB* (15), *bldC* (16), *bldH* (17,18), *whiG* (19) and *whiB* (19) were grown on R2YE or MS (mannitol soy flour) agar media, or minimal agar medium supplemented with 0.5% w/v mannitol (20) for 12–72 h (as indicated), or in liquid minimal medium (MM) supplemented with 0.5% w/v mannitol. All *Streptomyces* cultures were grown at 30°C. *Escherichia coli* strains were grown at 37°C in Luria-Bertani (LB) liquid or on LB solid medium. *Streptomyces* strains used in this study are summarized in Table 1.

RNA isolation

RNA was isolated as described previously (21); however, plate grown cultures were harvested by directly scraping cells from agar, overlaid with cellophane discs, into modified Kirby's mixture [1% w/v N-lauroylsarcosine sodium salt, 6% w/v sodium 4-amino salicylate, 6% v/v phenol mixture (pH 7.9) made in 50 mM Tris (pH 8.3)]. Total RNA samples were quantified by UV spectroscopy using the Ultrospec 3100 pro (Biochrom), and RNA quality was assessed using agarose gel electrophoresis.

RNA detection

Total RNA was detected using either SYBR Gold (Molecular Probes) staining or 3' pCp end-labeling with T4 RNA Ligase (Roche). Samples were run on 12% denaturing polyacrylamide gels, and were visualized using either UV light or autoradiography, respectively.

Oligonucleotides

The sequences of all DNA oligonucleotides used in this study, as well as the hybrid RNA/DNA adaptor used for RNA cloning, are summarized in Table 2.

RNA cloning

Total RNA (~100 µg) was separated on a denaturing 12% polyacrylamide gel and the region corresponding to the abundant 30–35 nt RNA species was excised. RNA was eluted from the gel slice overnight at 4°C in 20 mM Tris (pH 8.0), 0.5% SDS, 1 mM EDTA, and 0.4 M sodium acetate. Cloning of the RNA species was carried out using the method described by Lau *et al.* (22), with minor modifications. Briefly, RNA was recovered from the eluate by ethanol precipitation with 20 µg of glycogen, and samples were re-suspended in 30 µl dH₂O. Ligation of the 3' adapter (Modban; Table 2) was carried out for 1 h at 37°C in a 20 µl reaction volume consisting of 13 µl purified RNA, 10 µM 3' DNA adapter oligonucleotide (IDT DNA Technologies), 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 10 mM DTT, 60 µg/ml BSA, 10% DMSO, 40 U T4 RNA Ligase (Amersham Biosciences). The ligation mixture was ethanol precipitated, and separated on a 12% denaturing polyacrylamide gel followed by purification of the ligated products as described above, except that the RNA was resuspended in 20 µl dH₂O. The 3'-adapter-ligated RNA was then ligated to the DNA/RNA 5'-adapter (Nelson's linker; IDT DNA Technologies; Table 2) as described above, with the inclusion of 1 mM ATP. The ligation mixture was again ethanol precipitated and separated on a 12% denaturing polyacrylamide gel prior to purification. The RNA was then used as a template for reverse transcription (RT) using 200 U SuperScript II reverse transcriptase (Invitrogen) and the BanOne oligonucleotide (Table 2), whose sequence corresponded to the inverse complement of the 3'-adapter. The RT reaction was carried out at 42°C for 1 h. The resulting cDNA was used as template for PCR amplification with *Taq* DNA polymerase (Invitrogen) using oligonucleotides BanOne and BanTwo (Table 2). Products ~70 bp in size were recovered from a 2% agarose gel using a gel extraction kit (Qiagen) and

Table 2. Linker and oligonucleotide sequences used in this study

Linker/oligo name	Sequence (5' to 3')	Function
BanOne	ATTGATGGTGCCTACAG	Primer for reverse transcription and PCR amplification of cDNA
BanTwo	ATCGTAGGCACCTGAAA	Primer for PCR amplification of cDNA
ModBan	AMP-5'-p-5'p/CTGTAGGCACCATCAATdi-deoxyC-3'	3' Adaptor for RNA cloning
Nelson's linker	5'-ATCGTaggcaccugaaa-3' ^a	5' Adaptor for RNA cloning
M13 forward	GTAAAACGACGGCCAG	Sequencing of cDNA clones
M13 reverse	CAGGAAACAGCTATGAC	Sequencing of cDNA clones
5' Ala	GCTCTACCAACTGAGCTATAGCCCC	Northern blot analysis
3' Ala	GAATTGAACTCCTGACCTCTGCATGCCATGC	Northern blot analysis
5' Asn	GCCGGCTGCTCTGCCAATTGAGCTACCG	Northern blot analysis
5' His	AACCAGGTGCTCTACCAGCTGAGCTACACCCAC	Northern blot analysis
3' His	GTGAGTGACGGGACTTGAACCCGCGGCATCCTG	Northern blot analysis
5' Leu	CGCCGTGTCTGCATTCCACCATCCGGG	Northern blot analysis
3' Leu	GGACTTGAACCGGCACGCCCTCGAAGGGGCGAGC	Northern blot analysis
5' Met	CCCAGCGAGCTACCGAGCTGCTCCACCCC	Northern blot analysis
3' Met	CGGGGACAGGATTGAATCTGCGACCTCTG	Northern blot analysis
5' Ser	AGTGGCGGCCATAGACCGGACTAGGCGACGCCTCC	Northern blot analysis
3' Ser	GATTTGAACCCACGGTGACTTGCGCCACGAC	Northern blot analysis
3' Trp	CTTGAACCCCAACCGCTGGTTTGG	Northern blot analysis

^aThe sequence written in lower case represents RNA.

were directly cloned into the pCR[®]2.1-TOPO[®] vector (Invitrogen). Plasmid DNA was isolated from positive clones, and was sequenced using M13 forward and M13 reverse oligonucleotides (Table 2). The resulting sequences were then analyzed using BLAST (23).

Northern analysis

Total RNA samples were separated on 12% denaturing polyacrylamide gels and transferred to Zeta-Probe nylon membranes (BioRad) using a Trans-Blot semi-dry transfer cell (BioRad) (25 V for 30 min). Membranes were cross-linked using an XL-1000 UV crosslinker (Spectronics). 5' end-labeled oligonucleotides, corresponding to either the 5' or the 3' half of the tRNA of interest, were hybridized with the membranes overnight at 42°C in ULTRAhyb-oligo hybridization buffer (Ambion). Membranes were washed twice with 2 × SSC, 0.1% SDS for 30 min, followed by a single wash with 0.2 × SSC, 0.1% SDS for 10 min. Detection and quantification of signals were achieved using a Storm 820 phosphorimager (Molecular Dynamics) and ImageQuant v 5.2 (Molecular Dynamics) software. Where applicable, the change in the ratio of full-length tRNA:tRNA half was determined using intensity values obtained from the 'Volume Report' function of this software. To correct for background signals, we subtracted the intensities of equally sized areas on the blot, but adjacent to the bands, from all data points before using the resultant values for determining the ratio.

Translational inhibition and stringent response assay

Wild type *S. coelicolor* strain M145 was grown in 20 ml liquid MM supplemented with 0.5% w/v mannitol for ~40 h at 30°C. Cultures were then homogenized using a glass homogenizer before adding the indicated antibiotic or chemical (spectinomycin [200 µg/ml], hygromycin [50 µg/ml], thiostrepton [50 µg/ml], serine hydroxamate [SHX; 25 mM]). Cultures were then incubated for 30, 60 or 90 min, after which RNA was isolated as described above.

RESULTS

Detection of medium-dependent 30–35 nt RNA species

The initial goal of this work was to characterize small regulatory RNAs from *S. coelicolor*. To this end, we 3' end-labeled total RNA isolated from cultures grown for 24 or 48 h on either solid R2YE (rich) or MS media, and size-fractionated this labeled RNA on a 12% denaturing polyacrylamide gel. R2YE is a standard rich medium for *Streptomyces* growth (containing both glucose and yeast extract), while MS was developed as a sporulation medium (24), and contains a poorly utilized carbon source (mannitol). We observed a distinct population of 30–35 nt RNAs in the RNA samples isolated exclusively from cultures grown on MS medium, but not from those grown on rich medium (Figure 1a). This small RNA subpopulation was most abundant at 24 h, but was still detectable in the 48 h sample. To further explore the extent of this differential abundance, we examined RNA isolated from *S. coelicolor* cultures grown on MS medium over a 72 h developmental time course, which encompassed vegetative growth, aerial hyphae formation, and sporulation (Figure 1b). We discovered that these RNAs were not ubiquitously present in all RNA samples: they first appeared at times corresponding to the initiation of aerial hyphae formation (24 h) and then decreased in abundance as development proceeded through to late sporulation (Figure 1b). We also examined RNA isolated from cultures grown on rich R2YE medium over the same 72 h time course, and found that there were no 30–35 nt RNA species detectable at any time point, irrespective of the developmental stage (data not shown).

Cloning of 30–35 nt RNAs reveals an abundance of tRNA halves

To investigate the nature of this RNA population, we size-fractionated RNA isolated from cultures grown for 24 h on MS medium, and excised and purified the 30–35 nt

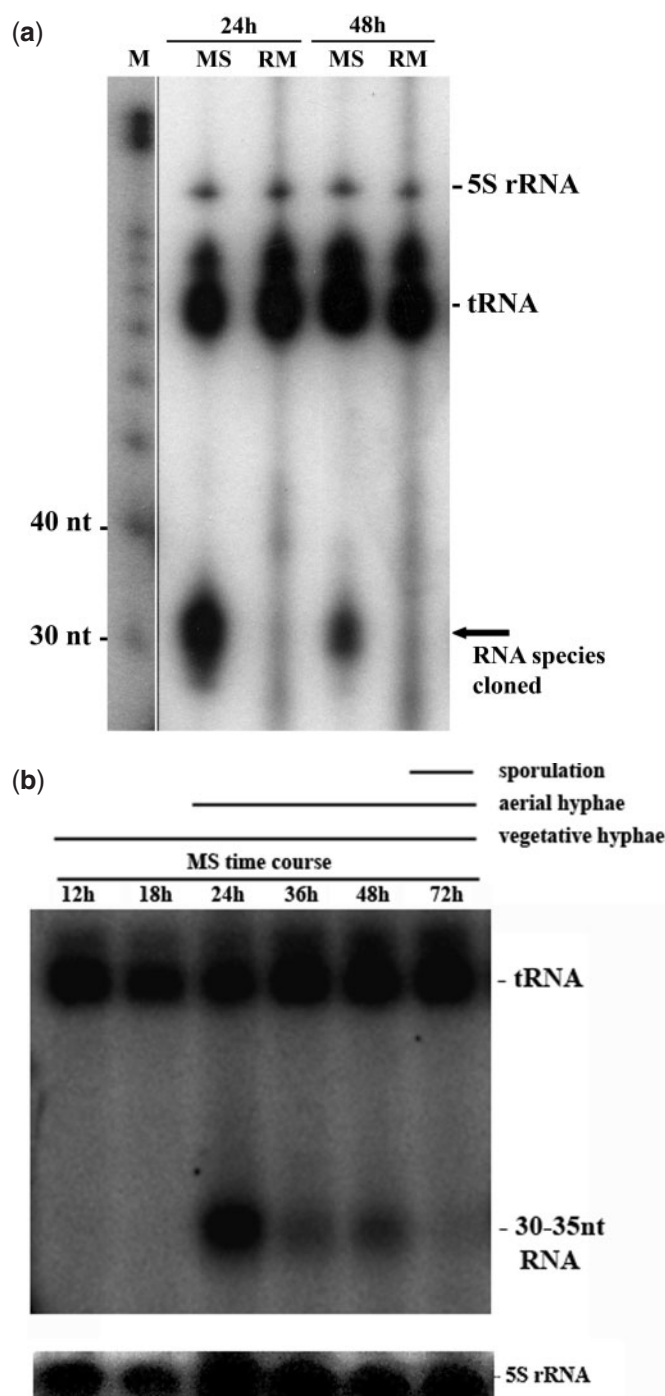


Figure 1. (a) A novel ~30–35 nt RNA species is detectable on MS, but not rich media. Total RNA harvested from MS and rich (R2YE) media after 24 or 48 h was labeled with pCp. Samples were separated on a 12% denaturing acrylamide gel and exposed to X-ray film. Distinct 30–35 nt RNA species were detectable only in samples isolated from MS medium-grown cultures. M: Decade marker (Ambion); MS: soy flour-mannitol medium, RM: Rich (R2YE) medium. (b) The appearance of a ~30–35 nt RNA species is temporally correlated with aerial development. A time course of 3' end-labeled RNA harvested from MS medium was separated on a 12% denaturing acrylamide gel. Shown in the *top panel* is full length tRNAs, together with the 30–35 nt region of the acrylamide gel, and in the *bottom panel* is the 5S RNA region of the gel, which was used as a control for RNA loading and RNA integrity.

RNAs. 5' and 3' linkers were ligated onto the ends of the purified RNA molecules to facilitate RT and PCR amplification of the resulting cDNAs, which were then cloned and sequenced. The majority of cloned sequences corresponded to tRNA genes (72%) (Supplementary Table 1), while 25% of the cloned intergenic sequences matched rRNA or signal recognition particle (SRP) RNA degradation products. Intriguingly, the cloned tRNA gene sequences did not appear to result from the random degradation of tRNAs, but instead corresponded to 'halves' of tRNAs located either 5' or 3' of the anticodon sequence. Halves corresponding to 17 different tRNAs were identified in our cDNA library; however, the number of clones for each tRNA varied significantly (Table 3). When multiple clones were obtained for a particular tRNA half, we frequently observed heterogeneity at both ends of the cloned sequences (Supplementary Table 1). This suggested that either cleavage was not occurring at a specific sequence, but rather in the general vicinity of the anticodon loop, or that the products of the initial cleavage event were targeted by 5' and 3' exonucleases.

There seemed to be no bias towards the cloning of 5' halves (10 different tRNAs) or 3' halves (9 different tRNAs); however, both halves were cloned for only one tRNA (Table 3). There was also no correlation between the number of genes encoding a particular tRNA and the number of clones obtained. We did, however, detect a significant codon-usage bias, as more clones of frequently used tRNAs (146/170 – primarily those used most frequently) were obtained when compared with those used less frequently (24/170, predominantly those used least frequently), based upon the statistics of *S. coelicolor* codon usage provided by The Institute for Genomic Research (http://www.gem.re.kr/tigr-scripts/CMR2/codon_tables.spl?project=ntsc02).

We also found that for the majority of cloned 3' halves, there was evidence supporting the existence of a terminal CCA sequence, with either the entire CCA sequence being present or some portion of it (Supplementary Table 1). The terminal CCA sequence is added post-transcriptionally to the majority of tRNAs in *S. coelicolor* (25), in contrast to the situation in *E. coli*, where the terminal CCA is encoded within all tRNA genes (26). This suggested that the tRNA cleavage products were derived from mature tRNAs that were capable of being aminoacylated, and not from immature/precursor tRNA molecules.

Global cleavage of tRNAs within the anticodon loop generates 30–35 nt stable RNA species

Given that the majority of sequenced cDNA clones corresponded to tRNA halves, we wanted to determine whether these could be correlated with the 30–35 nt species observed in the RNA-labeling experiments, as it was possible that their abundance represented a cloning artefact stemming from preferential RT and/or amplification of tRNAs relative to other RNAs/cDNAs. We carried out northern blot hybridisation experiments using RNA separated on a denaturing polyacrylamide gel, and probed for an individual tRNA half using oligonucleotides specific for the 5' or 3' end of the tRNA^{His}_{GUG}. We found

the appearance of the 30–35 nt RNA species could be directly correlated with the appearance of both 5' and 3' His tRNA halves (Figure 2). To investigate whether the appearance of tRNA halves was limited to particular tRNAs or whether it represented a more global phenomenon, we carried out northern blot analysis using a variety of probes for tRNA halves including: 5' and 3' Met (cloned many corresponding 5' halves but no 3' halves); 5' Asn (cloned many corresponding 3' halves); 3' Ser (cloned many corresponding 5' halves); and both 5' and 3' tRNA^{Leu}_{UUA} (which is the least used tRNA/codon in *S. coelicolor*, and is encoded by the *bldA* gene) (Figure 3). We detected tRNA halves corresponding to all of the 5' tRNA halves examined (Figure 3), including the *bldA* tRNA, suggesting that cleavage within the anticodon loop is a general phenomenon in *S. coelicolor*. Interestingly, we did not observe any 3' halves for Met, Leu or Ser tRNAs (Figure 3), despite having detected their corresponding 5' halves through either cloning experiments or northern blot analysis. This observation could not be extended to all 3' halves, however, as a number of sequences corresponding to 3' halves had been cloned in our initial investigation and were also detected by northern blot analysis (Figure 2 and data not shown). This implied that the 3' halves for particular tRNAs were significantly less stable than their 5' half counterpart.

tRNA cleavage profiles in developmental (*bld*) mutants are distinct from wild-type strains

The timing of tRNA cleavage, and the accumulation of the 30–35 nt RNA species on MS medium, coincided with both the initiation of aerial hyphae formation and the production of antibiotics in *S. coelicolor*. As this morphological and physiological transition is controlled by the *bld* genes, we were curious to see whether tRNA cleavage would be affected by mutations in these genes, and chose to focus on two of the tRNA halves for which

we had cloned numerous cDNA sequences (5' His and 5' Met; Table 3) and had examined previously in the wild-type strain (Figures 2 and 3). We isolated RNA from four different *bld* mutants (*bldA*, *B*, *C* and *H*) grown on MS medium. *bldC* and *bldH* both encode DNA-binding transcription factors (16,27); *bldB* encodes a small actinomycete-specific protein of unknown function (28,29), while *bldA* encodes the only tRNA specific for the rare Leu^{UUA} codon (30,31). We found that for all *bld* strains examined, the 5' His tRNA halves accumulated with a slight delay relative to that observed for the wild-type strain (Figures 2 and 4): halves were detectable by 48 h in all *bld* mutants, and reached maximal levels at 72 h. This could be due to slower growth rates of the *bld* mutants, relative to the wild-type strain, in this experiment. Interestingly, the halves were predominantly degraded by 96 h in *bldA*, *bldC* and *bldH*, but were still readily detectable in a *bldB* mutant (Figure 4). In contrast, however, a very different result was obtained when probing with the 5'-specific Met oligonucleotide. In the wild-type strain, 5' Met tRNA halves reached maximal abundance at 48 h, and were still readily detectable at 72 h (Figure 3). For *bldB*, a similar profile was observed (although this showed a delay relative to the wild type; Figure 4), but for *bldA*, *bldC* and *bldH*, Met-specific halves were not observed at any time point (Figure 4).

We also examined the appearance of tRNA halves in several mutants that were capable of raising aerial hyphae and producing antibiotics, but were blocked in their ability to form spore chains. These strains, referred to as *whi* mutants due to their white colony appearance, had tRNA cleavage profiles that closely resembled those of the wild-type strain for both 5' Met and 5' His tRNA halves (data not shown).

Taken together, these results demonstrate that *bld* (but not *whi*) mutants have patterns of tRNA cleavage and subsequent degradation that differ from the wild type, and from each other in the case of *bldB*. Given that *bld* mutants are defective in their ability to raise aerial hyphae (particularly *bldB* in this instance—see discussion below), these results would suggest that the appearance of tRNA

Table 3. Summary of cloned tRNA halves

tRNA half	Amino Acid	Anticodon	# cloned	% of tRNAs cloned
3'	Arg	CCT	8	4.7%
3'	Asn	GTT	23	13.5%
5'	Gln	CTG	9	5.3%
3'	Glu	CTC	3	1.8%
3'	Glu	TTC	2	1.2%
5'	Gly	GCC	1	0.6%
5'	His	GTG	13	7.6%
3'	His	GTG	36	21.2%
5'	Leu	GAG	6	3.5%
5'	Leu	CAG	1	0.6%
5'	Leu	TAG	1	0.6%
3'	Lys	CTT	1	0.6%
5'	Met	CAT	34	20.0%
5'	Phe	GAA	1	0.6%
5'	Pro	CGG	6	3.5%
5'	Ser	TGA	21	12.4%
3'	Thr	GGT	2	1.2%
3'	Val	CAC	2	1.2%
170				

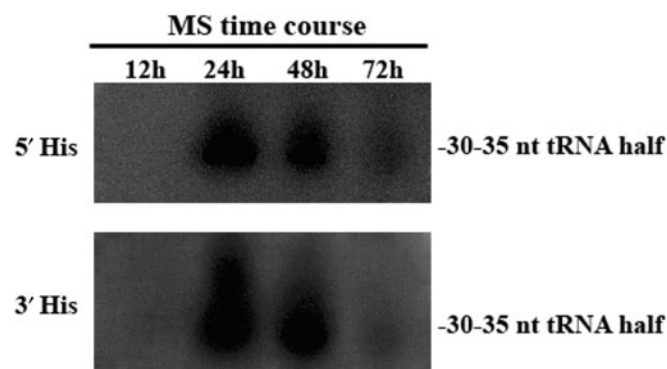


Figure 2. Northern blot analysis for the 5' and 3' halves of histidine tRNA in a wild-type background. Total RNA samples harvested from MS medium were separated on 12% polyacrylamide gels and were subjected to northern blotting using probes complementary to the 5' and 3' halves of the histidine tRNA. Blots were exposed to a phosphorimager.

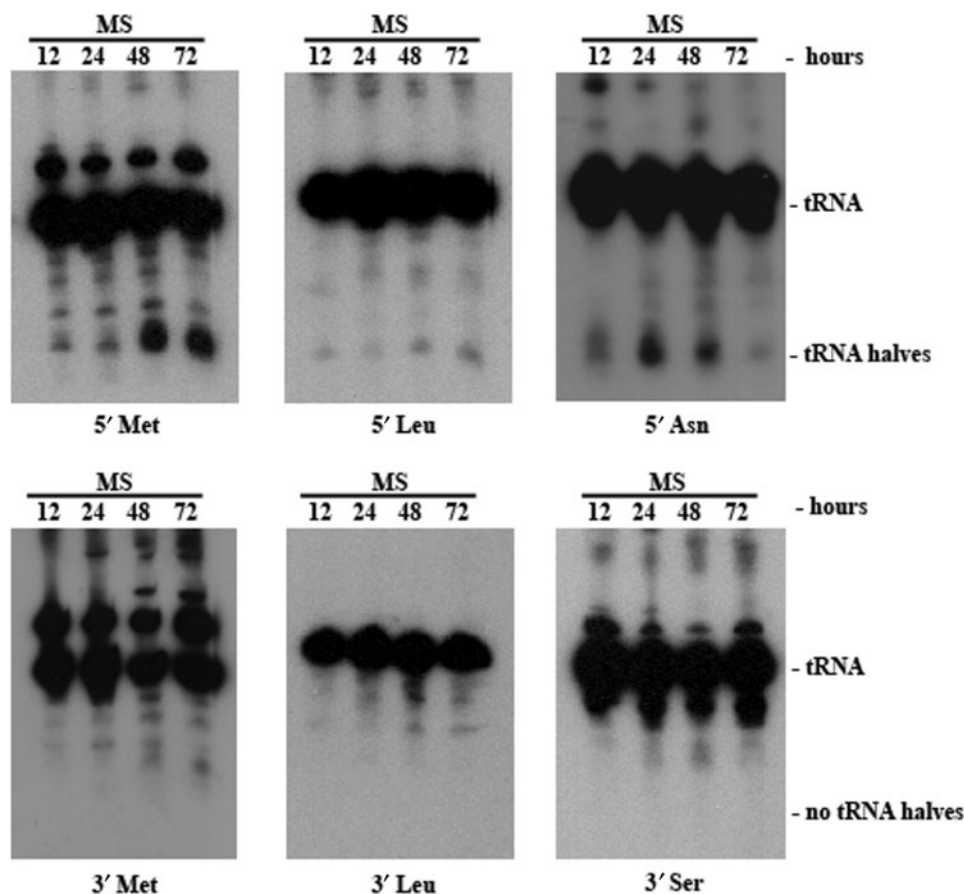


Figure 3. Northern blot analysis of tRNAs isolated from MS medium grown cultures. Total RNA was isolated from MS medium-grown cultures after 12, 24, 48 and 72 h of growth. The RNA was separated on 12% polyacrylamide gels and was subjected to northern blotting using probes that were complementary to the indicated tRNA half before being exposed to X-ray film.

halves is not dependent upon the initiation of aerial hyphae formation, antibiotic production or sporulation.

tRNA cleavage is not dependent upon the stringent response or ribosome inhibition

A similar tRNA cleavage phenomenon had recently been observed in *Tetrahymena* in response to amino acid deprivation (32), and we were curious about whether tRNA half accumulation in *S. coelicolor* could be correlated with amino acid limitation or deprivation of other nutrients. We had determined that tRNA halves could be detected in MS-grown strains (Figures 2–4); however, the composition of MS medium is not well defined (the major ingredient is soya flour), so we examined tRNA half accumulation in strains grown on a defined MM (20), where the only amino acid present was asparagine, which was provided as a nitrogen source. We observed an identical pattern of accumulation to that which we observed on MS medium (data not shown). We supplemented MM with yeast extract and proline, both individually and together, as these are the major source of amino acids added to the rich R2YE medium where tRNA halves were not detected. We did not observe a measurable decrease in tRNA halves, or even a delay in their appearance with any amino acid supplementation

(data not shown), suggesting that amino acid starvation may not be the primary factor stimulating tRNA cleavage in *S. coelicolor*. To determine whether carbon source influenced the appearance of tRNA halves, we replaced the mannitol in MS medium (a poorly utilized carbon source) with glucose (which is the primary carbon source included in the rich R2YE medium). The growth and development of *S. coelicolor* on the mannitol and glucose containing media was comparable, and, as for the amino acid supplementation, we still observed an accumulation of tRNA halves in the RNA harvested from glucose-grown cultures (data not shown). We also examined the effect of high osmolarity on the accumulation of tRNA halves, by adding sucrose (to a final concentration of 10.3%, as is found in R2YE) to MS medium, and found that tRNA halves accumulated in the same manner as in a low-osmolarity medium. Finally, we investigated the effects of buffering the medium by adding TES buffer to MS medium. Studies have shown that vegetative growth is associated with increased acidification of unbuffered media, which the wild type, but not *bld* mutants, are capable of neutralizing during aerial hyphae formation (33). As for all other supplementation experiments, we saw no difference in tRNA half accumulation relative to that of the unbuffered control strain (data not shown).

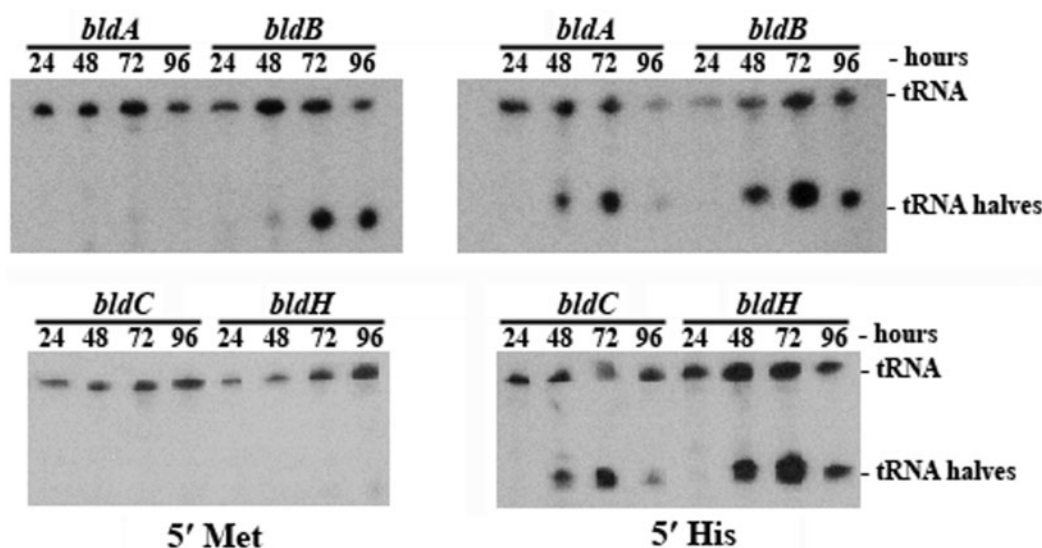


Figure 4. Northern blot analysis of tRNAs isolated from *bld* mutants. Total RNA from *bldA*, *bldB*, *bldC* and *bldH* mutant strains was isolated from MS-grown cultures after 24, 48, 72 and 96 h of growth, as indicated. The RNA was separated on 12% polyacrylamide gels, and was subjected to northern blotting using probes complementary to the 5' half of Met (left panels) or His (right panels) tRNA.

Given our inability to prevent the cleavage of tRNAs through medium supplementation, we decided to assess whether we could stimulate tRNA cleavage. The initiation of aerial hyphae formation and antibiotic production has been linked to the stringent response in a number of *Streptomyces* species (34,35,8). The stringent response can be induced either by amino acid starvation or by exposure to SHX, where SHX is a serine analogue that prevents the charging of serine tRNAs through the competitive inhibition of seryl tRNA synthetases (36). We added SHX to liquid MM cultures that had been grown for ~40 h, and continued growth for an additional 30, 60 or 90 min. While we saw a general trend towards increasing tRNA halves for 5' His (Figure 5), 5' Met and 5' Ser (data not shown) compared with an untreated control, this difference was not statistically significant. Similarly, we tested the effects of three antibiotics that specifically target the ribosome, and thus would arrest translation, to determine whether a decrease in translation (and hence decreased requirement for tRNAs) could stimulate tRNA cleavage. Spectinomycin targets the 16S rRNA within the 30S ribosomal subunit, preventing the translocation of the charged tRNA from the A-site to the P-site of the ribosome (37,38); thiostrepton changes the conformation of ribosomal protein L11 and is thought to hinder the binding of elongation factors (39); while hygromycin primarily interacts with 16S rRNA and inhibits ribosomal translocation (40). As was observed for the SHX treatment, addition of spectinomycin and thiostrepton caused a small increase in tRNA half abundance; however, again, this was not statistically significant after averaging the results of three independent biological trials (Figure 5). Hygromycin, on the other hand, caused a significant decrease in detectable tRNA halves (Figure 5), despite sharing ribosome-inhibitory characteristics with spectinomycin and thiostrepton.

DISCUSSION

We have discovered a unique tRNA cleavage event in the filamentous bacterium *S. coelicolor*, the timing of which coincides with both morphological differentiation and antibiotic production. Intriguingly, medium composition dictated whether tRNA halves were detected: tRNA halves were observed in RNA isolated from cultures grown on MM/MS medium (irrespective of carbon source), but not on rich medium. Previous work by Lee and Collins (32) showed that in *Tetrahymena thermophila*, tRNA cleavage within the anticodon loop (generating tRNA halves equivalent to those observed here) occurred in response to amino acid starvation. Propagation of *T. thermophila* requires a growth medium supplemented with 10 essential amino acids, and removal of even one of these from the growth medium was sufficient to stimulate a general tRNA cleavage response. In contrast, wild type *S. coelicolor* is capable of synthesizing all amino acids. We found that medium supplementation with additional amino acids had no effect on the appearance or accumulation of tRNA halves, suggesting that cleavage in *S. coelicolor* does not occur solely in response to amino acid starvation, and may instead occur in response to additional or alternative nutrient limitations. Intriguingly, tRNA cleavage has now also been observed in yeast during entry into stationary phase or during stress-induced apoptosis (D. Thompson and R. Parker, personal communication), suggesting that this phenomenon may be an evolutionarily conserved response to specific stresses.

bld mutants are defective in their ability to raise aerial hyphae, and in many cases, in their ability to produce antibiotics as well. These defects in aerial hyphae formation can, in some cases, be rescued by growth on alternative carbon sources such as mannitol (as is found in MS and minimal media), although carbon source has no effect on antibiotic production defects. Given the timing of tRNA

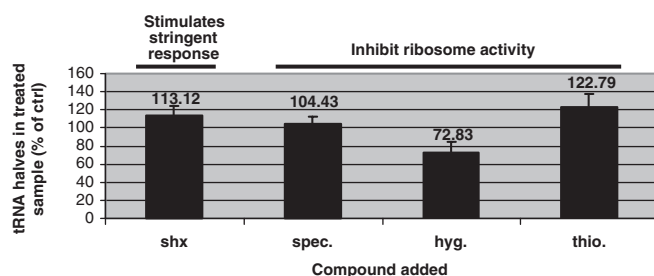


Figure 5. Translational inhibition and stringent response assay. Cultures were grown for ~40 h in liquid MM at 30°C before the addition of the indicated compound (shx = 25 mM serine hydroxamate; spec = 200 µg/ml spectinomycin; hyg = 50 µg/ml hygromycin; thio = 50 µg/ml thiostrepton) and incubation for an additional 60 min. Total RNA was then harvested and tRNA halves were detected using northern blot hybridization. The change in the ratio of tRNA halves in treated samples to untreated controls was determined by quantifying the appropriate bands after normalizing for background signal. The data shown are the average of results from three independent experiments. Error bars indicate one standard deviation from the mean.

half appearance, we examined the tRNA profiles of several *bld* mutants to see whether defects in morphogenesis and physiology affected the pattern of tRNA half appearance. There appeared to be two distinct *bld* mutant tRNA profiles: the *bldB* mutant profile looked very similar to that of the wild type, while those of *bldA*, *bldC* and *bldH* were very similar to each other, but did not show the same global cleavage pattern seen in the wild type. Interestingly, *bldB* is the only mutant of these four whose developmental defects cannot be rescued by growth on alternative carbon sources. As the *bldB* tRNA cleavage profile was most like that of the wild type, this suggests that aerial hyphae formation is not a pre-requisite for tRNA cleavage (as *bldB* mutants fail to raise aerial hyphae on MS medium), and that tRNA cleavage is not a sufficient signal to stimulate aerial hyphae formation. Furthermore, as *bldA*, *bldC* and *bldH* mutants can form a modest aerial mycelium on MS medium, but have altered tRNA cleavage patterns, this suggests that normal tRNA cleavage is also not necessary for aerial hyphae formation. While it cannot be formally excluded that the different tRNA cleavage patterns in these four *bld* mutant strains are due to differing genetic backgrounds, it seems unlikely given that very different cleavage profiles were seen for two *bld* mutants having the same genetic background (*bldB* and *bldC*).

The generation of the tRNA halves appeared to result from a cleavage event occurring within the anticodon loop. Given the single-stranded nature of this loop region, it could serve as a possible substrate for the single stranded-specific endonuclease RNase E (41). Interestingly, in *S. coelicolor*, RNase E activity is upregulated during development (42), at a time that coincides with the appearance of tRNA halves, and thus could be responsible for the observed cleavage of tRNAs. This upregulation of RNase E activity was absent in a *bldA* mutant but was not affected by a mutation in *bldC* (42). As the tRNA cleavage profiles appeared to be virtually identical for *bldA* and *bldC* mutants, this suggests that the upregulation of RNase E activity does not play a major role in either

the initial tRNA cleavage event or the ultimate degradation of the cleavage products. Unlike the global tRNA cleavage that we have observed here, cleavage of specific tRNAs has been observed previously in *E. coli* (43,44). *E. coli* produces several molecules having extremely specific tRNA cleavage capabilities: colicin D cleaves Arg tRNAs in their anticodon loop (43), while colicin E5 cleaves Tyr, His, Asn and Asp tRNAs in their anticodon loops (44). These colicins differ from each other both mechanistically, and at a sequence/structure level, and BLAST searches suggest that there are no similar proteins found in *S. coelicolor* (data not shown).

tRNAs undergo significant processing during their maturation into functional molecules. Mature 5' ends are generated by a conserved RNase P cleavage event, whilst the generation of mature 3' ends is far less conserved, even amongst bacteria; it may involve the concerted effort of several exonucleases and endonucleases such as RNase E, RNase III, and the recently discovered RNase Z (for which there is no obvious homologue in *S. coelicolor*) (45). In eukaryotes, the generation of full length tRNAs may also require the removal of introns through a splicing event that generates tRNA halves, which are then ligated together. There is no evidence for tRNA introns in *S. coelicolor*, and thus it seems unlikely that the tRNA halves observed are an intermediate in the synthesis process. Further supporting this is the observation that most of the 3' halves cloned showed evidence of 3' processing and addition of the terminal CCA sequence, which is the final step in tRNA processing and maturation.

The fate of the resulting 5' and 3' tRNA halves is clearly different in many instances. Strikingly, we were unable to detect 3' halves for a number of tRNAs despite observing 5' halves, in some cases in significant abundance (e.g. 5' Met). This suggested that the 3' halves might either be more susceptible to degradation, or are being preferentially degraded relative to the 5' halves. One possible mechanism to explain this preferential degradation would be through the activity of a 5' → 3' exonuclease. Such activity has recently been demonstrated in *Bacillus subtilis* by an enzyme termed RNase J1 (46), which has a role in the maturation of 16S rRNA. RNase J1 has a demonstrated preference for single-stranded 5' ends, particularly 5' monophosphorylated substrates, (which often result from endonucleolytic cleavage events) or 5' hydroxyl groups (which may be the product of spontaneous 'in-line cleavage' or cleavage by a single strand-specific endonuclease), but not primary transcripts having a 5' triphosphorylated end (46). Cleavage within the anticodon loop would result in exposure of a single-stranded 5' end of the 3' half, which could then serve as a substrate for an RNase J1-like enzyme. In contrast, the 5' end of the 5' half would be protected from such nuclease activity through its base-pairing to the acceptor stem portion of the 3' half, and would only become accessible when this base-pairing was disrupted through degradation of the 3' half. There is an RNase J1 homologue in *S. coelicolor* (SCO5745), the gene for which is located immediately upstream of a ribosomal RNA gene cluster. This protein is predicted to have ~40% amino acid identity (60% similarity) to the *B. subtilis* RNase J1 protein, suggesting that it likely shares a similar function, although this has yet to be tested experimentally.

The biological function of tRNA cleavage in *S. coelicolor*, and other organisms, is not immediately obvious. It is conceivable that reducing the number of tRNAs available for translation would contribute to a slowing down of protein synthesis. Our results from the pCp-labeling experiments, shown in Figure 1b, reveal that at 24 h, the 30–35 nt RNA species (which likely represents all tRNA halves) appears to be as abundant as the full length tRNAs; this would be expected to have a considerable effect on the translational capability of the colony at this time point. This effect could be amplified by decreasing the availability of highly used tRNAs, as is suggested by the bias in cloning of sequences corresponding to tRNAs that specify highly used codons. Such a slow down in protein synthesis has been observed in *S. coelicolor* liquid cultures, and has also been suggested to occur during the transition from vegetative growth to aerial hyphae formation on solid medium (47). tRNA cleavage may, therefore, act as a metabolic cue to switch developmental programs during growth on MM. The medium dependence of tRNA half accumulation is intriguing, although not unprecedented in *S. coelicolor*, as the expression of a number of key morphogenetic proteins can differ significantly depending upon the growth medium (48,49). The absence of detectable tRNA halves on rich medium may suggest that different signals contribute to the switching of developmental programs relative to what is seen on MM. Alternatively, tRNA cleavage may also be occurring during growth on rich medium, but the subsequent degradation of the resulting halves may be too rapid to permit detection within the time courses examined.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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REFERENCES

1. Elliot, M.A., Buttner, M.J. and Nodwell, J.R. (2007) Multicellular development in *Streptomyces*. In Whitworth, D.E (ed.), *Myxobacteria: Multicellularity and Differentiation*. ASM Press, Washington, D.C., pp. 419–438.
2. Claessen, D., de Jong, W., Dijkhuizen, L. and Wosten, H.A.B. (2006) Regulation of *Streptomyces* development: reach for the sky! *Trends Microbiol.*, **14**, 313–319.
3. Kelemen, G.H. and Buttner, M.J. (1998) Initiation of aerial mycelium formation in *Streptomyces*. *Curr. Opin. Microbiol.*, **1**, 656–662.
4. Pope, M.K., Green, B.D. and Westpheling, J. (1996) The *bld* mutants of *Streptomyces coelicolor* are defective in the regulation of carbon utilization, morphogenesis and cell-cell signalling. *Mol. Microbiol.*, **19**, 747–756.
5. Rigali, S., Nothaft, H., Noens, E.E.E., Schlicht, M., Colson, S., Müller, M., Joris, B., Koerten, H.K., Hopwood, D.A. et al. (2006) The sugar phosphotransferase system of *Streptomyces coelicolor* is regulated by the GntR-family regulator DasR and links N-acetylglucosamine metabolism to the control of development. *Mol. Microbiol.*, **61**, 1237–1251.
6. Viollier, P.H., Minas, W., Dale, G.E., Folcher, M. and Thompson, C.J. (2001) Role of acid metabolism in *Streptomyces coelicolor* morphological differentiation and antibiotic biosynthesis. *J. Bacteriol.*, **183**, 3184–3192.
7. Karandikar, A., Sharples, G.P. and Hobbs, G. (1997) Differentiation of *Streptomyces coelicolor* A3(2) under nitrate-limited conditions. *Microbiology*, **143**, 3581–3590.
8. Chakraborty, R. and Bibb, M. (1997) The ppGpp synthetase gene (*relA*) of *Streptomyces coelicolor* A3(2) plays a conditional role in antibiotic production and morphological differentiation. *J. Bacteriol.*, **179**, 5854–5861.
9. Chatterji, D. and Kumar Ojha, A. (2001) Revisiting the stringent response, ppGpp and starvation signaling. *Curr. Opin. Microbiol.*, **4**, 160–165.
10. Hesketh, A., Chen, W., Ryding, J., Chang, S. and Bibb, M. (2007) The global role of ppGpp synthesis in morphological differentiation and antibiotic production in *Streptomyces coelicolor* A3(2). *Genome Biol.*, **8**, R161.
11. Magnusson, L.U., Farewell, A. and Nyström, T. (2005) ppGpp: a global regulator in *Escherichia coli*. *Trends Microbiol.*, **13**, 236–242.
12. Deutscher, M.P. (2003) Degradation of stable RNA in bacteria. *J. Biol. Chem.*, **278**, 45041–45044.
13. Davis, B.D., Luger, S.M. and Tai, P.C. (1986) Role of ribosome degradation in the death of starved *Escherichia coli* cells. *J. Bacteriol.*, **166**, 439–445.
14. Piret, J.M. and Chater, K.F. (1985) Phage-mediated cloning of *bldA*, a region involved in *Streptomyces coelicolor* morphological development, and its analysis by genetic complementation. *J. Bacteriol.*, **163**, 965–972.
15. Merrick, M.J. (1976) A morphological and genetic mapping study of bald colony mutants of *Streptomyces coelicolor*. *J. Gen. Microbiol.*, **96**, 299–315.
16. Hunt, A.C., Servín-González, L., Kelemen, G.H. and Buttner, M.J. (2005) The *bldC* developmental locus of *Streptomyces coelicolor* encodes a member of a family of small DNA-binding proteins related to the DNA-binding domains of the MerR family. *J. Bacteriol.*, **187**, 716–728.
17. Champness, W.C. (1988) New loci required for *Streptomyces coelicolor* morphological and physiological differentiation. *J. Bacteriol.*, **170**, 1168–1174.
18. Chater, K.F., Bruton, C., King, A.A. and Suarez, J.E. (1982) The expression of *Streptomyces* and *Escherichia coli* drug-resistance determinants cloned into the *Streptomyces* phage phi C31. *Gene*, **19**, 21–32.
19. Flärdh, K., Findlay, K.C. and Chater, K.F. (1999) Association of early sporulation genes with suggested developmental decision points in *Streptomyces coelicolor* A3(2). *Microbiology*, **145**, 2229–2243.
20. Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F. and Hopwood, D.A. (2000) *Practical Streptomyces Genetics*. The John Innes Foundation, Norwich, U.K.
21. Hopwood, D.A., Bibb, M.J., Chater, K.F., Kieser, T., Bruton, C.J., Kieser, H.M., Lydiate, D.J., Smith, C.P., Ward, J.M. and Schrepf, H. (1985) *Genetic manipulation of Streptomyces – A laboratory manual*. The John Innes Foundation, Norwich, U.K.
22. Lau, N.C., Lim, L.P., Weinstein, E.G. and Bartel, D.P. (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science*, **294**, 858–862.
23. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.

24. Hobbs, G., Frazer, C.M., Gardner, D.C.J., Cullum, J.A. and Oliver, S.G. (1989) Dispersed growth of *Streptomyces* in liquid culture. *Appl. Microbiol. Biotechnol.*, **31**, 272–277.
25. Sedlmeier, R., Werner, T., Kieser, H.M., Hopwood, D.A. and Schmieger, H. (1994) tRNA genes of *Streptomyces lividans*: new sequences and comparison of structure and organization with those of other bacteria. *J. Bacteriol.*, **176**, 5550–5553.
26. Fournier, M.J. and Ozeki, H. (1985) Structure and organization of the transfer ribonucleic acid genes of *Escherichia coli* K-12. *Microbiol. Mol. Biol. Rev.*, **49**, 379–397.
27. Takano, E., Tao, M., Long, F., Bibb, M.J., Wang, L., Li, W., Buttner, M.J., Bibb, M.J., Deng, Z.X. and Chater, K.F. (2003) A rare leucine codon in *adpA* is implicated in the morphological defect of *bldA* mutants of *Streptomyces coelicolor*. *Mol. Microbiol.*, **50**, 475–486.
28. Pope, M.K., Green, B. and Westpheling, J. (1998) The *bldB* gene encodes a small protein required for morphogenesis, antibiotic production, and catabolite control in *Streptomyces coelicolor*. *J. Bacteriol.*, **180**, 1556–1562.
29. Eccleston, M., Ali, R.A., Seyler, R., Westpheling, J. and Nodwell, J. (2002) Structural and genetic analysis of the BldB protein of *Streptomyces coelicolor*. *J. Bacteriol.*, **184**, 4270–4276.
30. Lawlor, E.J., Baylis, H.A. and Chater, K.F. (1987) Pleiotropic morphological and antibiotic deficiencies result from mutations in a gene encoding a tRNA-like product in *Streptomyces coelicolor* A3(2). *Genes Dev.*, **1**, 1305–1310.
31. Leskiw, B.K., Bibb, M.J. and Chater, K.F. (1991) The use of a rare codon specifically during development? *Mol. Microbiol.*, **5**, 2861–2867.
32. Lee, S.R. and Collins, K. (2005) Starvation-induced cleavage of the tRNA anticodon loop in *Tetrahymena thermophila*. *J. Biol. Chem.*, **280**, 42744–42749.
33. Süssstrunk, U., Pidoux, J., Taubert, S., Ullmann, A. and Thompson, C.J. (1998) Pleiotropic effects of cAMP on germination, antibiotic biosynthesis and morphological development in *Streptomyces coelicolor*. *Mol. Microbiol.*, **30**, 33–46.
34. Ochi, K. (1986) Occurrence of the stringent response in *Streptomyces* sp. and its significance for the initiation of morphological and physiological differentiation. *J. Gen. Microbiol.*, **132**, 2621–2631.
35. Ochi, K. (1987) Metabolic initiation of differentiation and secondary metabolism by *Streptomyces griseus*: significance of the stringent response (ppGpp) and GTP content in relation to A factor. *J. Bacteriol.*, **169**, 3608–3616.
36. Tosa, T. and Pizer, L.I. (1971) Biochemical basis for the antimetabolite action of L-serine hydroxamate. *J. Bacteriol.*, **106**, 972–982.
37. Bilgin, N., Richter, A.A., Ehrenberg, M., Dahlberg, A.E. and Kurland, C.G. (1990) Ribosomal RNA and protein mutants resistant to spectinomycin. *EMBO J.*, **9**, 735–739.
38. Carter, A.P., Clemons, W.M., Brodersen, D.E., Morgan-Warren, R.J., Wimberly, B.T. and Ramakrishnan, V. (2000) Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature*, **407**, 340–348.
39. Jonker, H.R.A., Ilin, S., Grimm, S.K., Wöhnert, J. and Schwalbe, H. (2007) L11 domain rearrangement upon binding to RNA and thiostrepton studied by NMR spectroscopy. *Nucl. Acids Res.*, **35**, 441–454.
40. Pfister, P., Risch, M., Brodersen, D.E. and Böttger, E.C. (2003) Role of 16S rRNA helix 44 in ribosomal resistance to hygromycin B. *Antimicrob. Agents Chemother.*, **47**, 1496–1502.
41. Cohen, S.N. and McDowell, K.J. (1997) RNase E: still a wonderfully mysterious enzyme. *Mol. Microbiol.*, **23**, 1099–1106.
42. Hagège, J.M. and Cohen, S.N. (1997) A developmentally regulated *Streptomyces* endoribonuclease resembles ribonuclease E of *Escherichia coli*. *Mol. Microbiol.*, **25**, 1077–1090.
43. Tomita, K., Ogawa, T., Uozumi, T., Watanabe, K. and Masaki, H. (2000) A cytotoxic ribonuclease which specifically cleaves four isoaccepting arginine tRNAs at their anticodon loops. *PNAS*, **97**, 8278–8283.
44. Ogawa, T., Tomita, K., Ueda, T., Watanabe, K., Uozumi, T. and Masaki, H. (1999) A cytotoxic ribonuclease targeting specific transfer RNA anticodons. *Science*, **283**, 2097–2100.
45. Redko, Y., Li de la Sierra-Gallay, I. and Condon, C. (2007) When all's zed and done: the structure and function of RNase Z in prokaryotes. *Nat. Rev. Microbiol.*, **5**, 278–286.
46. Mathy, N., Benard, L., Pellegrini, O., Daou, R., Wen, T. and Condon, C. (2007) 5'-to-3' exoribonuclease activity in bacteria: role of RNase J1 in rRNA maturation and 5' stability of mRNA. *Cell*, **129**, 681–692.
47. Novotna, J., Vohradsky, J., Berndt, P., Gramajo, H., Langen, H., Li, X.M., Minas, W., Orsaria, L., Roeder, D. and Thompson, C.J. (2003) Proteomic studies of diauxic lag in the differentiating prokaryote *Streptomyces coelicolor* reveal a regulatory network of stress-induced proteins and central metabolic enzymes. *Mol. Microbiol.*, **48**, 1289–1303.
48. Willey, J., Santamaria, R., Guijarro, J., Geistlich, M. and Losick, R. (1991) Extracellular complementation of a developmental mutation implicates a small sporulation protein in aerial mycelium formation by *S. coelicolor*. *Cell*, **65**, 641–650.
49. Capstick, D.S., Willey, J.M., Buttner, M.J. and Elliot, M.A. (2007) SapB and the chaplins: connections between morphogenetic proteins in *Streptomyces coelicolor*. *Mol. Microbiol.*, **64**, 602–613.